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ORIGIN AND FUNCTION OF THE PROTOPLASMIC CONSTITUENTS IN PELOMYXA CAROLINENSIS

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Introduction

The protoplasmic constituents of several species of *Pelomyxa* have been described by Greeff (1874), Gould (1894), Wilson (1900) and Wilber (1942) and some suggestions have been made concerning their origin and their function but none of these suggestions are supported by experimental evidence. It, therefore, seems desirable to study the origin and function of these structures experimentally.

Relative Specific Gravity of the Protoplasmic Constituents

Open bottom hematocrit tubes were made of pyrex glass. The smaller opening was closed with a rubber band and the tubes were half filled with a heavy solution of gum-arabic in culture fluid. Then about 1 cc. of culture fluid, containing 5 or 10 pelomyxae, was carefully added and the solutions at the interface stirred with a glass rod and mixed so as to produce in this region a gradient of density and viscosity. The tubes were then put into an electric centrifuge and rotated, after which the rubber bands were quickly removed and the contents poured into partially frozen culture fluid, so as to prevent recovery of the pelomyxae. Some of these centrifuged, chilled pelomyxae were put into each of the following: one per cent osmic acid in culture fluid, ninety-five per cent alcohol, Champy's fluid, Regaud bichromate-formol mixture, and Bouin's fluid. These preparations were then studied under high and low magnifications. The results obtained are presented in Figure 1 and the following paragraphs.

Figure 1 shows that the pelomyxae during centrifugation were much elongated, that the refractive bodies (Wilber, 1942) aggregated at one end and the fat globules and contractile vacuoles at the other and that the food-vacuoles, the nuclei, the crystals, the beta granules, and the clear hyaloplasm respectively in fairly definite layers between; but it does not show which end is heavier.

Numerous specimens were consequently withdrawn from the tubes immediately after centrifugation, by means of an ice-cold capillary pipette of such small bore

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that they could not turn end for end. The capillary was then immersed in nujol, to eliminate distortion caused by the curved side walls, and examined under the compound microscope. Invariably, the refractive bodies were at the centrifugal and the fat globules at the centripetal end. The refractive bodies, consequently,

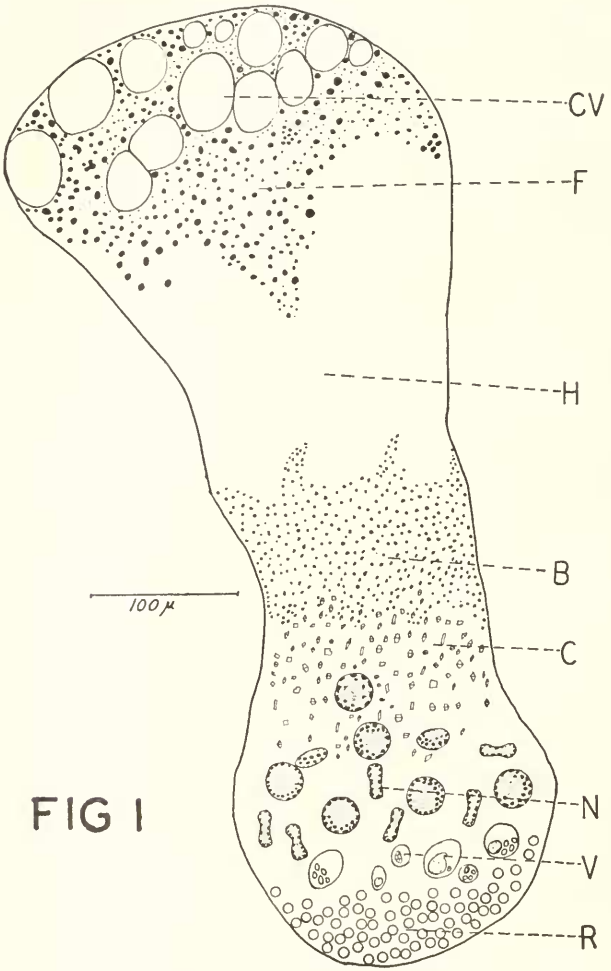


FIG 1

FIGURE 1. Camera sketch of a centrifuged *Pelomyxa* showing stratification of the protoplasmic constituents. R, refractive bodies (at centrifugal end); V, food-vacuoles; N, nuclei; C, crystals; B, beta granules (mitochondria); H, layer containing only hyaloplasm; F, fat; Cv, contractile vacuoles.

are the heaviest and the fat globules and contractile vacuoles the lightest constituents of *Pelomyxae carolinensis*. Mast and Doyle (1935) obtained similar results in observations on *Amoeba proteus*. However the substance in the nuclei did not stratify as in *Amoeba proteus*, indicating that either all the nuclear material has the

same specific gravity or that the viscosity of it is so high that the granules do not stratify under the centrifugation used.

The crystals were free in the cytoplasm, and not in vacuoles as occurs very commonly in uncentrifuged specimens and in centrifuged amoebae (Mast and Doyle), and many of them were broken probably by striking one another during centrifugation. Apparently, at the centrifugation used the crystals were thrown out of the vacuoles and aggregated in a different layer.

Recovery of Centrifuged Pelomyxae

Pelomyxae were centrifuged as described above and put on slides and examined under high magnification while the temperature slowly increased to that of the room. As the temperature increased, the plasmasol (Wilber, 1942) began to flow toward the centripetal end in all but a few specimens in which very small, stubby pseudopods first formed at the centrifugal end but these were soon withdrawn and the plasmasol began to flow in the opposite direction. The refractive bodies were the first protoplasmic constituents to enter the flowing plasmasol. They were carried toward the centripetal end and became distributed in the lower layer of the plasmasol. After the refractive bodies were all in the stream the food-vacuoles were carried forward and came to lie above the refractive bodies; then the crystals and beta granules were carried forward. In this manner the protoplasmic constituents were redistributed through the cell. The pelomyxae remained in the monopodal form during and for some time after the redistribution. The entire process required about 2 minutes.

Function of Nuclei

Pelomyxae were mounted on a slide without a cover-slip, examined under the high power of the binocular dissecting microscope, and left undisturbed until they had attached and had extended one or more pseudopods. Then, with the aid of a fine glass needle, pseudopods which contained refractive bodies, crystals, beta granules, and contractile vacuoles, but only a few or no nuclei, were cut off and mounted on a slide under a cover-slip supported by a ring of vaseline, some with numerous chilomonads, others with no food. The slides were examined under the low and high power of the compound microscope to ascertain the effect of removing various numbers of nuclei, on locomotion, feeding, digestion, and longevity.

Locomotion: After the operation, most of the fragments remained quiet for 30 to 120 minutes. Then all except those with no nuclei became active and soon moved precisely like normal specimens in all respects, and sudden increase in the intensity of light caused them to stop moving, just as it does normal specimens. Moreover, the method of formation of pseudopods was the same as in normal pelomyxae except that those with less than 9 or 10 nuclei invariably assumed the monopodal form which is not characteristic of uninjured pelomyxae.

All the anucleate fragments were sluggish. Coarsely granular material was aggregated in the center leaving a peripheral layer with no cytoplasmic constituents except beta granules; but usually there was no clear hyaline layer, although some few did have a distinct hyaline cap. The process of locomotion was greatly modified. In fact, most of the fragments did not move at all unless stimulated strongly.

These results are like those obtained by Verworn (1909), Gruber (1912), Stolć (1910) and Willis (1916) in observations on *Amoeba*.

Feeding and Digestion: Numerous fragments obtained as described above were examined carefully. All nucleate fragments ingested food and the process was the same as in uninjured pelomyxae. Fragments with 1, 3, 6, and 10 nuclei respectively were stained with neutral red and the process of digestion carefully observed. No difference from the normal was evident. Refractive bodies were formed as in normal specimens. The increase in number observed in 36 hours after feeding is given in Table I. This table indicates that the number of refractive bodies formed varied inversely with the number of nuclei.

TABLE I

Table showing the relation between the number of nuclei and the increase in number of refractive bodies in nucleate fragments of *Pelomyxa carolinensis*.

Number of nuclei in each fragment	Number of refractive bodies		Increase in per cent	Average
	Before feeding	36 hours later		
1	10	16	60	63
1	3	5	66+	
3	9	13	44+	45
3	4	5	25	
3	6	10	66+	
6	12	17	41+	39
6	8	11	37+	
10	31	30	0	0

Ten anucleate fragments were studied. Seven of these did not ingest anything; three ingested one chilomonad each. One of these was observed during the process of ingestion. It formed a food-cup which was apparently normal. The ingested chilomonads died in the food-vacuoles but were not digested. One of the fragments was sketched 24 hours after it had ingested a chilomonad (Fig. 2F). The chilomonad was then dead but still intact and there was no evidence of digestion even after 72 hours. A partially digested chilomonad was in a food-vacuole in this fragment at the time of operation; digestion proceeded no further (Fig. 2F′).

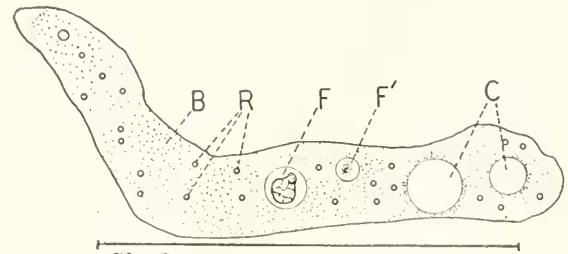
These results indicate that anucleate fragments of *Pelomyxa* sometimes ingest food and kill it but that they do not digest it, or food which was ingested before the operation. The results agree in part with those of Stolć (1910) who maintains that anucleate amoebae ingest food normally for several days. They are not in accord with the views of Hofer (1890), Gruber (1912), Willis (1916), Lynch (1919), and Becker (1926) who contend that anucleate amoebae do not ingest food. Becker, however, maintains that in anucleate fragments of *Amoeba dubia* "Food once ingested can be digested by the amoebae after the fashion of normal amoebae."

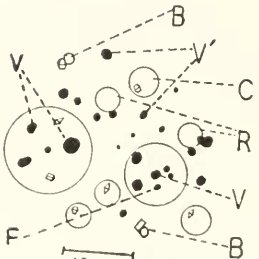
Length of life: Numerous fragments with different numbers of nuclei but with the same number of beta granules, crystals, and refractive bodies (Wilber, 1942) were obtained as described above. Great care was taken to have the volume of the various fragments as nearly equal as possible. They were all passed through five

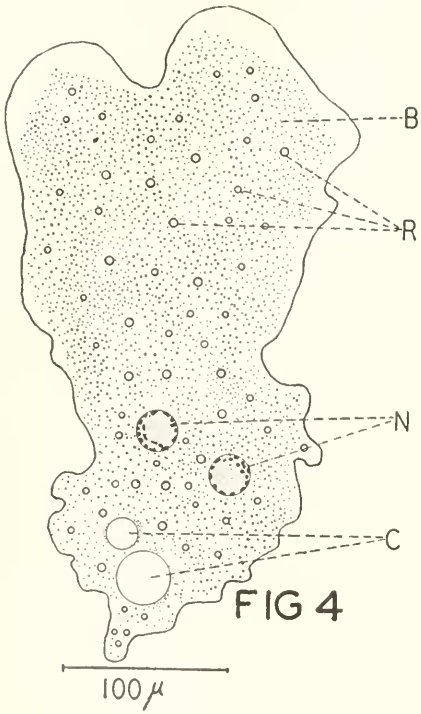
TABLE II

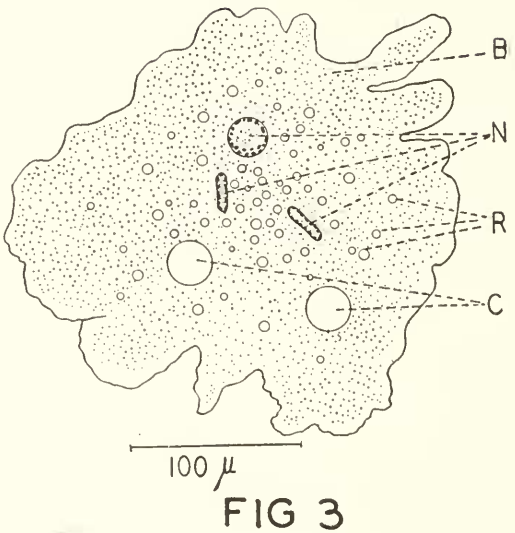
Table showing the relation between the number of nuclei and the length of life in fragments of *Pelomyxa carolinensis* in the absence of food.

Number of nuclei	Number of food-vacuoles	Number of refractive bodies	Average	Length of life in days	Average
1	0	10		8	
1	1	3	6.2	4	6.2
1	0	5		6	
1	1	7		7	
2	2	3		8	
2	0	8	5.6	6	6.3
2	0	6		5	
3	0	4	7.0	5	7.5
3	2	10		10	
4	1	12		6	
4	2	3	6.0	10	8.5
4	2	3		10	
4	0	6		8	
5	1	4		8	
5	9	12	8.0	7	8.3
5	1	8		10	
8	2	12		19	
8	1	8	11.0	23	19.0
8	3	13		15	
10	2	14		17	
10	1	12	12.3	14	18.6
10	3	11		25	
12	1	15	16.0	21	20.0
12	3	17		19	
20	3	20		23	
20	3	19	18.6	19	24.0
20	3	17		30	
25	2	21	18.5	26	23.5
25	1	16		21	
40	2	16	23.0	21	24.0
40	2	30		27	
50	4	18		27	
50	4	10	22.6	29	28.6
50	3	40		30	









FIGURES 2–5.

FIGURE 2. Camera sketch of anucleate fragment of *Pelomyxa carolinensis*. C, contractile vacuoles; F, food-vacuole containing a chilomonad which was ingested after all the nuclei had been removed; F¹, food-vacuole present before the operation; R, refractive bodies; B, beta granules.

FIGURE 3. Camera sketch of a fragment of a pelomyxa one minute after it had been cut off. B, beta granules; C, contractile vacuoles; N, nuclei; R, refractive bodies. The fragment had no contractile vacuoles when it was cut off. Note that one minute later, two contractile vacuoles had formed, but that there was no aggregation of beta granules around them. They contracted about one hour after the sketch was made but there was no indication of aggregation even then.

FIGURE 4. Camera sketch of a fragment of a pelomyxa one hour after it had been cut off. N, nuclei; C, contractile vacuoles; R, refractive bodies; B, beta granules. The fragment had no contractile vacuoles until shortly before the sketch was made. Note that a few beta granules

separate 10 cc. portions of sterile culture fluid, then each was put into 5 cc. of this fluid in a small glass dish and observations made on the relation between the number of nuclei and the length of life.

When the fragments failed to respond to stimulation with a fine glass rod, they were tentatively considered dead. When they showed evidence of disintegration, they were pronounced dead. The occurrence of these two phenomena never differed by more than four hours. The results obtained are given in Table II. This table shows that the fragments with one to 5 nuclei lived roughly the same length of time, but that those with more than 5 nuclei lived much longer.

It may be contended that this is due to differences in the number of refractive bodies and food-vacuoles in the fragments or to differences in their size. As stated above, all the fragments were nearly the same in size. The difference in the length of life observed could therefore not have been due to difference in size. The table shows that while in the fragments with 5 or fewer nuclei the length of life usually varied directly with the number of refractive bodies and food-vacuoles, in those with more than 5 nuclei this did not obtain. This seems to show that while the length of life of fragments of *Pelomyxa* is, at least under some conditions, dependent upon the number of refractive bodies and food-vacuoles present, it is also dependent upon the number of nuclei present.

The evidence presented, then, seems to show that the presence of at least one nucleus is necessary for locomotion and digestion but not for ingestion of food, and that the length of life of a fragment, without food, varies directly with the number of nuclei and the number of food-vacuoles and refractive bodies present.

Formation of the Contractile Vacuoles and the Relation Between Them and the Beta Granules

Numerous pelomyxae were examined under the high power of a binocular dissecting microscope. The animals were left undisturbed on a slide without a cover-slip until they had attached and had extended one or more pseudopods. With the aid of a fine glass rod, pseudopods which contained one or more nuclei, refractive bodies, crystals, and beta granules, but *no contractile vacuoles,* were cut off and mounted on a slide under a cover-slip supported by a ring of vaseline, and examined under the oil immersion objective of a compound microscope.

The following was observed: New contractile vacuoles formed in all the fragments. The time for the formation of new contractile vacuoles varied from about 60 seconds to more than one hour after the operation (Fig. 3). The vacuole was first visible as a small fluid-filled space in the hyaloplasm, no larger than a beta granule. This grew rapidly in size until it was about 5 micra in diameter and then enlarged more slowly until it contracted. At no time was there an aggregation

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have aggregated around them. Ten minutes after this sketch was made, the two vacuoles coalesced and another one formed between the two nuclei.

Figure 5. Camera sketch of portion of cytoplasm in a starved pelomyxa twenty-four hours after it had ingested a colpidium. F, food-vacuoles; V, vacuole-refractive bodies in the food-vacuoles; V′, vacuole-refractive bodies in the cytoplasm; B, bleb with a crystal attached to it; C, crystal in a vacuole; R, vacuole-refractive bodies in contact with blebs. Note that some of the food-vacuoles contain crystals and vacuole-refractive bodies. Some of the vacuole-refractive bodies are free in the cytoplasm. Several are in contact with blebs formed from the crystals. Note also two crystals with blebs and crystals in vacuoles.

of beta granules in the region where the vacuole formed but after it had discharged a few times, beta granules aggregated around it (Fig. 4).

The time required for the beta granules to aggregate around the contractile vacuole varied greatly. In some of the fragments studied, there was no sign of aggregation for several days after the vacuole had formed and begun to function; in others, aggregation began very soon after it had formed. The presence or absence of nuclei in the fragments had no apparent effect on the time of formation of the contractile vacuole and the variation in time was as great in anucleate as in nucleate fragments, and the nucleus had no effect on the time required for the aggregation of beta granules around the vacuole.

In uninjured pelomyxae, the cytoplasm immediately around the contractile vacuole is more viscous than that which is elsewhere in the plasmasol (Wilber, 1942). Young contractile vacuoles, however, i.e., vacuoles which had recently arisen *de novo,* were seen often to come into intimate contact with nuclei, food-vacuoles, and other structures in the cytoplasm, and the beta granules in the cytoplasm adjoining them had as marked Brownian movement as those elsewhere in the plasmasol. It was not until after the first contraction that evidence of higher viscosity was observed in the cytoplasm adjoining the surface of the newly formed vacuoles. These facts indicate that the cytoplasm adjoining the surface of the contractile vacuole does not become viscous until some time after the vacuole has arisen. After a viscous layer has formed around a contractile vacuole, the latter always expands in this viscous substance after each contraction. New vacuoles, however, arise elsewhere in the cytoplasm.

It thus appears that in *Pelomyxa* the beta granules are not directly related to the formation of contractile vacuoles and that their aggregation and the gelation of the cytoplasm around the contractile vacuoles are in no way necessary for the action of the vacuoles.

These results are in accord with the contention of Mast (1938), in reference to *Amoeba proteus* but not with those of Howland and Pollack (1927) in reference to *Amoeba verrucosa.* Mast says: "The differentiation of a layer of substance on the surface of the contractile vacuole is probably due to the action of the fluid in the vacuole on the adjoining cytoplasm," and "neither the beta granules nor the layer of viscous substance is involved in the function of the contractile vacuole." Howland and Pollack (1927) maintain that the gelated region around the contractile vacuole can "be considered as supplying the initial impulse for systole. . . ." This is not true in *Pelomyxa carolinensis* for in it the gelated layer does not form until after the first contraction. It is, therefore, not necessarily involved in the contraction of the vacuole and it obviously does not necessarily function in the accumulation of fluid in the vacuole.

It can, therefore, be concluded that neither the beta granules nor the viscous cytoplasm around the contractile vacuoles is of primary importance in the formation or the function of the contractile vacuoles.

Function of the Beta Granules

Wilber (1942) demonstrated that the beta granules in *Pelomyxa carolinensis* stain like mitochondria and he concludes: "These granules, like the beta granules of *Amoeba proteus* (Mast and Doyle, 1935), are consequently similar in composition to the mitochondria in metazoan cells." Metcalf (1910), referring to *Amoeba*

proteus, holds that they are involved in the excretion of substance by the contractile vacuole and he consequently calls them "excretory granules."

Mast and Doyle (1935) attempted to ascertain their function in *Amoeba proteus* by centrifuging and then cutting out different proportions and noting the effect and by observing their movements in normal specimens. They concluded that "the beta granules function in accumulation of fluid eliminated by the contractile vacuole." They are the only investigators who approached the problem experimentally.

In the present work, the experiments and observations of Mast and Doyle were repeated on *Pelomyxa carolinensis*. Some specimens were centrifuged and cut at different levels so as to remove various constituents and different proportions of the beta granules (Fig. 1). Others not centrifuged were cut, to obtain fragments of approximately the same size as the centrifuged fragments, but with normal proportions of all the protoplasmic constituents. Both kinds of fragments were mounted in culture fluid, without food, on slides under cover-slips supported by rings of

TABLE III

Table showing the effect of removing different protoplasmic constituents on the length of life of pelomyxae. F, fat; Cv, contractile vacuoles; H, hyaloplasm; B, beta granules.

Constituents removed	Number of specimens	Average length of life in days	
		Constituents removed	No constituents removed
F + Cv	10	18	21
F + Cv + ½H	9	7	22
F + Cv + H	10	1	19
F + Cv + H + ½B	8	1	20
F + Cv + H + B	8	¼	15

vaseline and studied until all the fragments had died. This was repeated five times. The results obtained in a typical experiment are presented in Table III.

Table III shows the following: The uncentrifuged fragments lived, on an average, from 15 to 22 days. The centrifuged fragments produced by cutting so as to remove the fat and contractile vacuoles lived nearly as long as the uncentrifuged fragments. Those produced by cutting so as to remove the fat, contractile vacuoles, and about half of the hyaloplasmic layer lived about one-third as long. Those produced by cutting so as to remove practically all the hyaloplasmic layer lived only about one-twentieth as long, but those produced by cutting so as to remove approximately half of and all the beta granules respectively showed no marked further decrease in length of life.

It is evident from these results that the removal of the fat and the contractile vacuoles causes but little, if any, decrease in length of life, that removal of a large proportion of the hyaloplasm, in addition to the fat and the contractile vacuoles, causes a pronounced decrease in the length of life, and that the removal of the beta granules in addition to the hyaloplasm, fat, and contractile vacuoles causes little, if any, further decrease in length of life. These facts indicate that length of life in fragments of *Pelomyxa* is primarily dependent on the amount of hyaloplasm in them, and only secondarily, if at all, on the number of beta granules.

The results are in harmony with the contention of Holter (1936) and Holter and Kopac (1937) that enzymes in amoebae and in marine ova are localized in the hyaloplasm and not in any formed bodies, and also with the views of Just (1939) in regard to the great importance of the ground cytoplasm in cellular function.

The facts presented, therefore, seem to indicate that beta granules (mitochondria) do not play an active part in cell functions and that their observed movements are merely a visible sign of submicroscopic changes in the hyaloplasm.

Formation of Refractive Bodies

About 100 pelomyxae were centrifuged and cut so as to remove most of the fat and all the refractive bodies (the fat was removed so that it could not serve as a source of food). Half of them were then put into each of two dishes containing sterile culture fluid and numerous colpidia added to the fluid in one of the dishes. In about 50 minutes all the specimens had recovered from the "shock" of the operations, as indicated by the facts that they moved normally and responded to increase in intensity of light by cessation of movement.

Two of the 50 pelomyxae without food died 20 hours after the operation; four of them lived until the 7th day; none divided. The average length of life was five days. No vacuole-refractive bodies (Wilber, 1942) or refractive bodies formed in any of them. Three of the 50 pelomyxae with food did not feed and died four hours after the operation. Each of the others ingested one or more colpidia. Twenty-four hours after feeding, there were numerous vacuole-refractive bodies in the food-vacuoles and some in the cytoplasm of every one of these specimens, and some of the food-vacuoles in a few of them contained crystals. These results show that food is necessary for the formation of vacuole-refractive bodies and of refractive bodies, and that the crystals are formed in the food-vacuoles. They also show that removal of the refractive bodies has no apparent injurious effects on pelomyxae. This is in accord with the views of Mast and Doyle (1935) who contend "that the removal of all the refractive bodies does not seriously interfere with the vital processes in *Amoeba*," but is not in accord with the views of Singh (1938) who maintains that specimens of *Amoeba proteus* with the refractive bodies removed appear "as if they were encysted until these bodies are formed," and that "as soon as these bodies are formed, the amoebae regain their normal shape and activity."

Ten pelomyxae were kept in sterile culture fluid for 15 days; then colpidia were added and each pelomyxa examined under low and high magnifications at intervals of one hour. The results obtained in observations on all these pelomyxae are in accord with the following detailed description concerning one which had ingested two colpidia: One hour after ingestion, the two colpidia were dead, but there was no evidence of digestion. Two hours after ingestion, both colpidia stained deeply red with neutral red, but the fluid in the food-vacuoles did not stain; the vacuolar membrane was very close to the pellicle of the colpidia. Three hours after ingestion, the colpidia stained deeply red in neutral red, and the fluid in the vacuoles, orange; the vacuoles had enlarged, probably as a result of fluid passing in from the cytoplasm. Four hours after ingestion, both food-vacuoles had divided; fragments of the colpidia in the four resulting vacuoles stained deeply red; two of the vacuoles contained vacuole-refractive bodies which stained intensely red in neutral red (one

vacuole had three; the other, one). Six hours after ingestion, there were six food-vacuoles present; all contained vacuole-refractive bodies; two contained hyaline nonstaining spherical bodies about 4 micra in diameter and of unknown composition. Twelve hours after ingestion, there were numerous vacuole-refractive bodies in each of the six vacuoles and several crystals in four of them; the other two contained no crystals; the vacuole-refractive bodies varied from 1 micron to about 6 micra in diameter; several were free in the cytoplasm; the manner in which they entered the cytoplasm was not ascertained. Twenty-four hours after ingestion, there were eight food-vacuoles present; five contained vacuole-refractive bodies and crystals, three, only vacuole-refractive bodies; there were numerous vacuole-

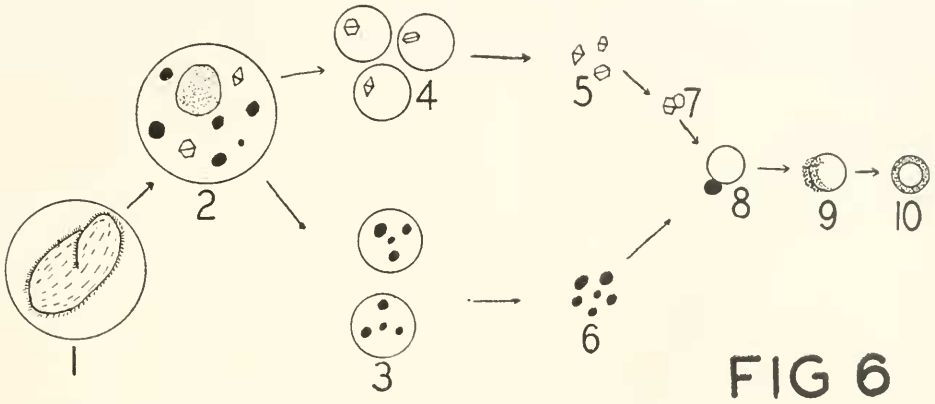

FIGURE 6. Diagram illustrating the formation of the refractive bodies from digested food. 1, food-vacuoles containing an ingested organism, usually a colpidium or a chilomonad; 2, food-vacuole containing a partially digested organism and crystals and vacuole-refractive bodies formed from the food during the process of digestion; 3 and 4, vacuoles formed by the division of 2; 5 and 6, crystals and vacuole-refractive bodies free in the cytoplasm; the manner in which they leave the vacuoles is not known; 7, crystal with bleb attached to it (the crystal disappears and the bleb greatly enlarges); 8, enlarged bleb in contact with a vacuole-refractive body; 9, vacuole-refractive body "flowing over" the bleb; 10, refractive body formed by the union of a bleb and a vacuole-refractive body. Stippling in 9 and 10 represents material of vacuole-refractive body.

refractive bodies and several crystals in the cytoplasm; some of the crystals had blebs attached (Fig. 5).

One of the crystals with a bleb attached was observed closely under oil immersion for several hours. The bleb became larger and simultaneously the crystal smaller until it was no longer visible. Soon another crystal came in contact with the bleb, and in about 40 minutes it too disappeared and the bleb became still larger. The crystals were obviously used in the growth of the bleb. Fifteen minutes later, the bleb came in contact with three vacuole-refractive bodies which were free in the cytoplasm. The substance in these three bodies "flowed over" the bleb and covered it with a layer, resulting in the formation of a body which was identical with other refractive bodies. The time required was 65 minutes. The beta granules took no visible part in the formation of the refractive body. Similar results were obtained in observations on crystals with blebs attached and vacuole-refractive bodies in normal pelomyxae.

The present observations clearly indicate that both the crystals and the vacuole-refractive bodies originate from the food in the food-vacuoles, that they leave the vacuoles and are found free in the cytoplasm (the manner in which this takes place is not known), and that the crystals form nonstaining spherical bodies (blebs) which become covered with a layer of substance from the vacuole-refractive bodies and thus form refractive bodies (Fig. 6).

The conclusions concerning the formation of the refractive bodies from the crystals and the vacuole-refractive bodies are in accord with the contention of Mast and Doyle (1935) who maintain that "The refractive bodies . . . originate and develop in the cytoplasm probably from substances obtained from the vacuole-refractive bodies and the crystals."

Mast and Doyle (1935) maintain that the beta granules function "in transferring digested substances from the food-vacuoles and the crystal vacuoles to the refractive bodies." There is no indication of such a function in *Pelomyxa*.

Function of the Refractive Bodies

Fifty pelomyxae were passed through five different portions of sterile culture fluid and then put into a stender dish containing 25 cc. of this fluid but no food. Each day a few were stained with neutral red, Sudan black, and Janus green B respectively, and these and a few unstained specimens examined for refractive bodies, fat, beta granules, and crystals. The results are presented in Table IV.

Table IV

Showing the decrease in number of refractive bodies in the absence of food.

Date	Number of refractive bodies in each of 9 pelomyxae									Average
	a	b	c	d	e	f	g	h	i	
Sept. 25	50	20	40	50	62	31	23	41	40	39.7
Sept. 30	42	18	32	—	50	27	17	34	30	31.2
Oct. 1	30	10	30	33	50	26	13	30	30	28.0
Oct. 2	23	10	27	27	—	26	13	30	23	22.3
Oct. 3	20	8	27	27	43	—	11	—	23	22.7
Oct. 4	17	8	23	24	43	23	11	25	20	21.5
Oct. 5	14	8	20	20	41	21	11	25	19	19.8
Oct. 10	14	8	17	20	37	15	7	21	10	16.4

This table shows that there is in *Pelomyxa carolinensis* a steady decrease in the number of refractive bodies during starvation. It was observed that some of the refractive bodies disintegrated and that during disintegration the outer layer gradually disappeared, leaving a homogeneous mass which did not stain neutral red. These results indicate that the refractive bodies function as reserve food. This conclusion is in accord with that of Mast and Doyle (1935) and Singh (1938) in regard to the function of the refractive bodies in *Amoeba proteus*.

Summary

1. Centrifugation causes the protoplasmic constituents of *Pelomyxa carolinensis* to stratify into definite layers which, in order of decrease in weight, are as follows:

refractive bodies, food-vacuoles, nuclei, crystals, beta granules, hyaloplasm, contractile vacuoles, and fat.

2. The presence of at least one nucleus is necessary for normal locomotion and digestion, but not for ingestion of food in *Pelomyxa*. The length of life of *Pelomyxa* during starvation varies directly with the number of nuclei present.

3. The contractile vacuoles arise *de novo* in the hyaline cytoplasm. Their formation and functioning is not dependent on the presence of nuclei or beta granules.

4. The beta granules (mitochondria) do not play an active role in cell functions but, probably, merely give visible evidence of submicroscopic changes taking place in the hyaloplasm.

5. The vacuole-refractive bodies and the crystals are formed from the food in the food-vacuoles of *Pelomyxa* and are used in the formation of the refractive bodies.

6. The refractive bodies function as food reserve in the cell. Their complete removal does not impair any of the cellular activities.

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